

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



(43) International Publication Date
18 March 2004 (18.03.2004)

PCT

(10) International Publication Number
WO 2004/022784 A3

(51) International Patent Classification⁷: C12Q 1/68

(21) International Application Number: PCT/SG2003/000209

(22) International Filing Date: 4 September 2003 (04.09.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data: 60/408,818 6 September 2002 (06.09.2002) US

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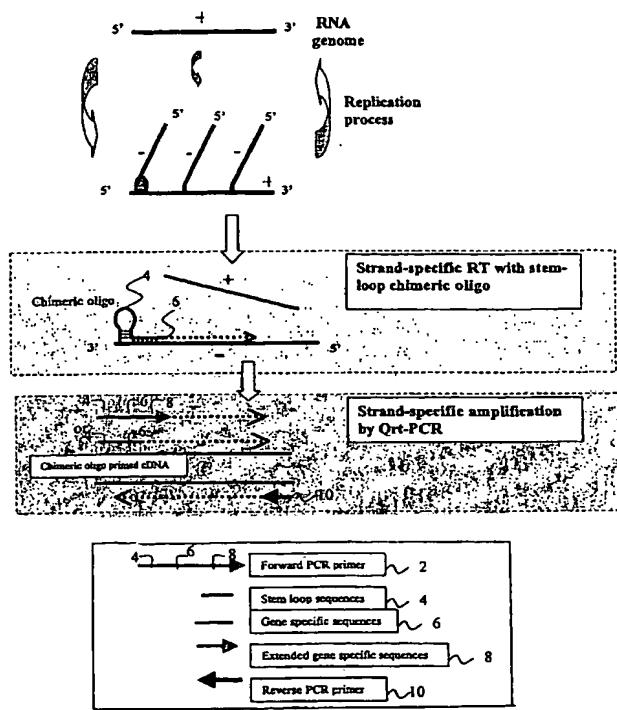
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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TI, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: STRAND SPECIFIC DETECTION AND QUANTIFICATION



WO 2004/022784 A3

(57) Abstract: An aspect of the present invention provides for specific compositions and methods of use make possible accurate, simple and efficient detection and quantification of desired nucleic acid strands. Teachings of the present invention also provide for novel and efficient primer design for annealing to specific target nucleic acid strands, as well as for designing particularly accurate amplification reaction primers for amplification of desired nucleic acid strands.



Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
21 May 2004

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/SG 03/00209A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02 057479 A (INVITROGEN CORP) 25 July 2002 (2002-07-25) examples 12,31,35	11-16, 22-24 1-10, 17-21
X	WO 96 01327 A (DAVID FABRICE ;LABIMAP SA (FR)) 18 January 1996 (1996-01-18) page 4-7; figures 2,5	11-16, 22-24 1-10, 17-21
X	US 6 117 635 A (NAZARENKO IRINA A ET AL) 12 September 2000 (2000-09-12) figures 1,9	11-16, 22-24 1-10, 17-21
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 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search.

Date of mailing of the international search report

16 March 2004

29/03/2004

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/SG 03/00209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 573 906 A (MULLER FRANCIS ET AL) 12 November 1996 (1996-11-12) column 2; figure 1	11-16, 22-24 1-10, 17-21
X	US 5 874 260 A (CLEUZIAT PHILIPPE ET AL) 23 February 1999 (1999-02-23) figure 1	11-16, 22-24
A	column 10; figure 9	1-10, 17-21
X	WO 98 49351 A (UNIV MASSACHUSETTS) 5-November-1998-(1998-11-05) abstract	1-24
X	NAZARENKO ET AL: "A CLOSED TUBE FORMAT FOR AMPLIFICATION AND DETECTION OF DNA BASED ENERGY TRANSFER" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 25, no. 12, 1997, pages 2516-2521, XP002094959 ISSN: 0305-1048	11-16, 22-24
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A	the whole document	1-10, 17-21
A	CRAGGS JOANNA K ET AL: "Development of a strand-specific RT-PCR based assay to detect the replicative form of hepatitis C virus RNA" JOURNAL OF VIROLOGICAL METHODS, vol. 94, no. 1-2, May 2001 (2001-05), pages 111-120, XP002273663 ISSN: 0166-0934 cited in the application the whole document	1-24
		-/-

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SG 03/00209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>LIN LAN ET AL: "A novel strand-specific RT-PCR for detection of hepatitis C virus negative-strand RNA (replicative intermediate): Evidence of absence or very low level of HCV replication in peripheral blood mononuclear cells" JOURNAL OF VIROLOGICAL METHODS, vol. 100, no. 1-2, February 2002 (2002-02), pages 97-105, XP002273664 ISSN: 0166-0934 cited in the application abstract</p> <p>---</p>	1-24
A	<p>SEIPP STEFANIE ET AL: "Establishment of a highly specific detection system for GB virus C (GBV-C) minus-strand RNA" VIRUS RESEARCH, vol. 56, no. 2, August 1998 (1998-08), pages 183-189, XP002273665 ISSN: 0168-1702 cited in the application abstract</p> <p>---</p>	1-24
A	<p>HOUNG HUO-SHU H ET AL: "Quantitative detection of dengue 2 virus using fluorogenic RT-PCR based on 3'-noncoding sequence" JOURNAL OF VIROLOGICAL METHODS, vol. 86, no. 1, April 2000 (2000-04), pages 1-11, XP002273666 ISSN: 0166-0934 the whole document</p> <p>---</p>	1-24
A	<p>VOS P ET AL: "AFLP: A NEW TECHNIQUE FOR DNA FINGERPRINTING" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 23, no. 21, 1995, pages 4407-4414, XP000939214 ISSN: 0305-1048 figure 1</p> <p>-----</p>	6-8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG 03/00209

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: 1-24; all partially because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box 1.2

Claims Nos.: 1-24; all partially

In view of the wording of the claims presently on file, which render it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of PCT Article 6 (Rule 6.1(a) PCT).

Terms like "convertible" or "hemi-nested primer", which apparently refer to essential features of the invention, have no technical meaning in the context of nucleic acid reactions. The step of "transcription" (i.e. the synthesis of an RNA molecule by means of a DNA-dependent RNA-polymerase using a DNA template) is not only inconsistent with the present description and examples, where no transcription reaction is shown, but also meaningless in the context of the present methods as claimed. The imprecise use of terms "G" (without reference to the reaction involved), or "Ta" and "Tm" (referring to nucleic acids and to reactions alike), and the vague expressions like "first conformation structure", "conditions of a first reaction", "differing reaction conditions", "transcription reaction conditions", or "amplification reaction conditions", are extremely vague and confusing. In addition, the wording of product claims in terms of process steps, or method claims whose purpose is not related to the steps carried out, renders the present claims so unclear that a meaningful search on the basis of the claims is impossible.

Consequently, the search has been carried out for those parts of the application which do appear to be clear, namely the methods explained in the description 74 to 162, and specially as summarized in 13 to 18 and the elements of Figures 2, 4, 5, 7 and 8). In other words:

- Methods of strand-specific RNA amplification comprising the steps of:

(i) Reverse Transcription of the RNA target using a bifunctional oligonucleotide primer having a 5' "tag" region which forms a stem-loop structure during the low temperature RT reaction and a linear structure during the high temperature PCR reactions, and a 3' target binding region (Figure 4; "convertible" oligonucleotide).

(ii) Real-time quantitative PCR amplification of the cDNA strand obtained in step 1 using (i) a target specific "reverse" primer (Figure 2, item 10); and (ii) a "forward" primer comprising the complete sequence of the bifunctional primer plus a short 3' extension of target-specific nucleotides (Figure 2, item 2; Figure 8; "hemi-nested primer").

- Bifunctional hairpin primer having a 5' "tag" region which forms a stem-loop structure and a 3' target-specific region.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/SG 03/00209

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 02057479	A	25-07-2002	CA	2433330 A1	25-07-2002
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